Study on Degradation of Common Pesticides by Selected Species of Soil –Borne Beneficial Bacteria

Synopsis

Submitted in the partial fulfilment for the requirement of

Doctor of Philosophy

in

Botany

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DAYALBAGH, AGRA-282005

2022

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1.0 INTRODUCTION

1.1 Pesticides

The word pesticides encompass a heterogeneous group of chemicals developed to control a variety of pests.Food and Agriculture Organization (FAO) of the United Nations defines, "*pesticide as any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances*."Pesticides contain chemicals that are used to kill various types of insects. It no longer requires an introduction, because it has become an integral part of our life. Pesticides are used to control various pests and disease carriers, such as mites, nematodes and rodents. Pesticides in agriculture are used to control weeds, insect infestation and diseases.

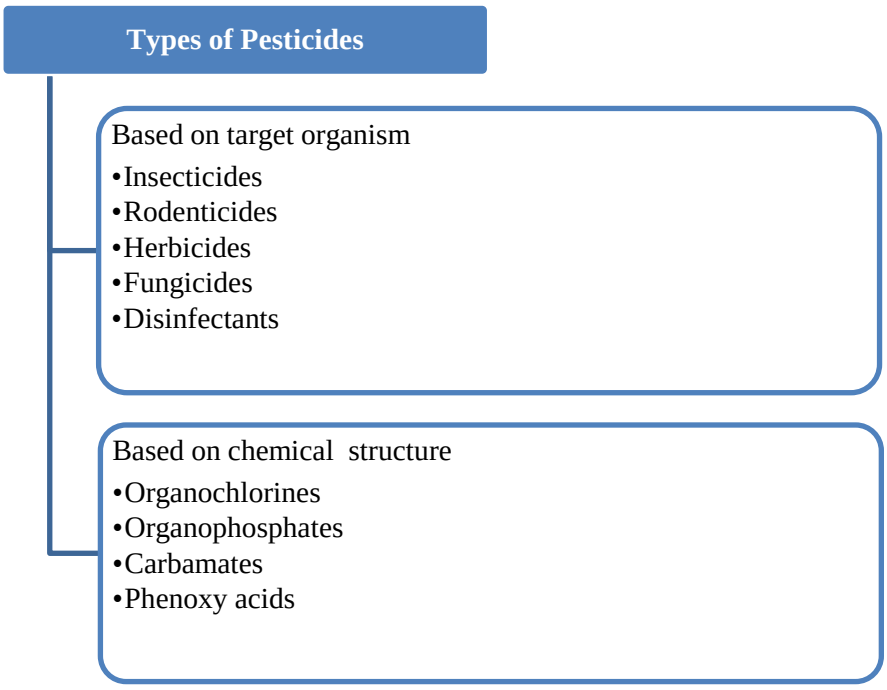

Fig. 1 Types of pesticides (Bohmont *et al* .,1997)

1.2 Global Scenario of Pesticide Consumption

The use of pesticides has increased in both developed and under developing countries during the last decades. The Figure 2.0 given below which show percentage share of pesticides consumption by different continent. This figure shows that Asia is largest consumer of pesticides.

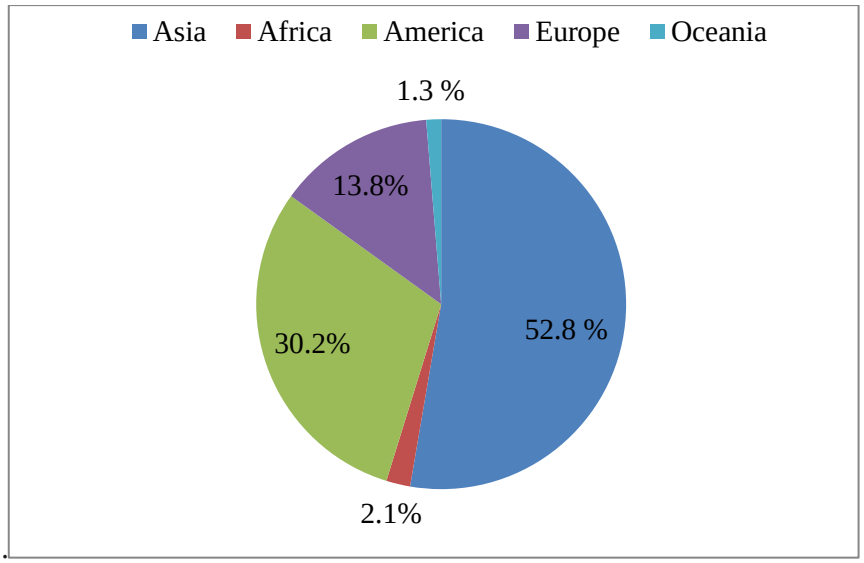

Fig. 2 Percentage share of pesticides consumption by different continent (FAO, 2019)

1.3 Pesticides Consumption in India 1955 to 2021

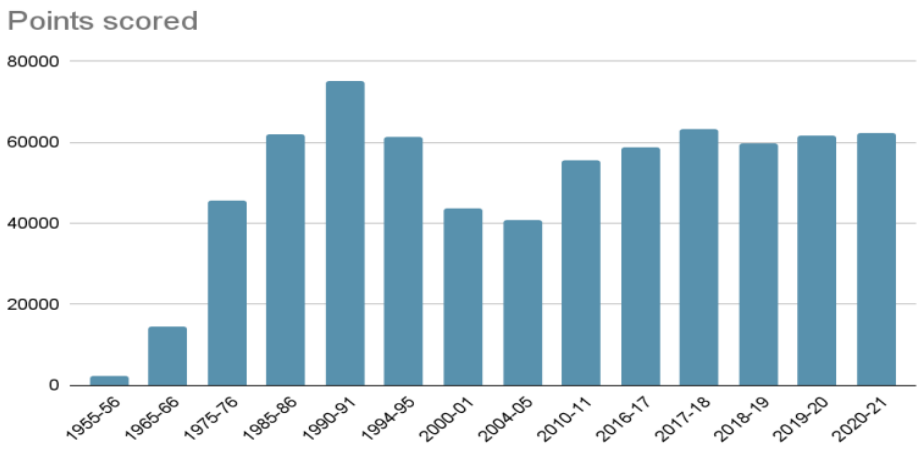

Fig. 3 Data on pesticides consumption in India over last years
(Source-Government ofIndia, Ministry of agriculture and farmers welfare ppqs.gov.in)

In India, the consumption of pesticides has increased logarithmically after green revolution. Pesticide consumption in the year 2010 was 55,542 MT which increased to 61702 MT in 2019-20and has further increased to 62193MT (GCA) in 2020-21. The green revolution led to introduction of pesticides and by adopting the pesticides farmers have been able to increase the crop yield.

1.4 Benefits of Pesticides

Pesticides help reduce vector borne and insect borne diseases and Herbicides have removed the hardship of hand weeding. Pesticides in a way have transformed developing countries into food producers.Table 1.0 summarizes primary and secondary benefits of pesticides ,primary benefits are

known as consequences of the direct pesticides' effects such as protection of crop health. The secondary benefits arise from primary.

Table 1 Benefits of pesticides

PRIMARY BENEFITS	SECONDARY BENEFITS
1.controlling pests and plant disease vectors • Invasive species controlled • Improved crop/livestock quality • Reduced soil disturbance	**1.Community benefits** • Food safety/security • Life expectancy increased • Reduced maintenance costs
2. Controlling disease vectors and nuisances organisms • Human disturbance reduced • Animal suffering reduced • Increased livestock quality	**2.National benefits** • National agricultural economy • Increased export revenues • Reduced soil erosion/moisture loss
3.control of organisms that harm other human activities and structures • Recreational turf protected • Wooden structures protected	**3.Global benefits** • Less pressure on uncropped land • Fewer pest introductions elsewhere

This has addressed the issue of Food Security, by increased availability and affordability of food to all, however as a net result one of the core key elements of food security, which is food safety has been compromised with.

1.5 Hazards of Pesticides Use

By the use of these pesticides we have not only gained benefits but also have introduced harmful effects. On human and other living beings long term low dose exposure to pesticides are increasingly linked to negative impacts .

1.5.1 Negative impacts on human health

Table 2 Negative impacts on human health

S. No.	Negative effects on human health	Reference
1	Immune suppression	Brouwer *et al.,* 1999; Crisp *et al*., 1998; Hurley *et al.,* 1998
2	Hormone disruption by mimicking or antagonising natural hormones in the body.	
3	Diminished intelligence	
4	Reproductive abnormalities	
5	Causes cancer	

1.5.2 Negative impacts on ecosystem

Pesticides' negative effects are not only limited to the human being, they also affecting other living beings, and the ecosystem. Day by day declining in the numbers of vultures (Plaza*et al.,* 2019) and bees (Johansen *et al.,* 1990) are considerable examples of ecosystem disturbance induced by pesticides .Herbicides may also hurt insects or spiders indirectly when they feed on the pesticide contaminated foliage that these animals need for their food and shelter. Non-target birds and rodents may also be killed if they ingest poisoned grains set out as bait for them (US EPA, 1998).Bats can accumulate high concentrations of Organo- chlorine (OCs) and may be affected by their potential toxic .

1.6 Role of Soil Microbiota

Microorganisms play an important role in many soil biological processes, including nitrogen transformations, organic matter decomposition, nutrient release and their availability, as well as in stabilizing the soil structure and affecting its fertility (Vyas *et al.,* 1988; Edwards *et al.,* 1990; Khan*et al.,* 2000). Soil microflora is the first biota that undergoes direct and indirect impacts of toxic substances introduced to soil. Due to its fast response to contaminants, ubiquity, size and recycling of elements soil microflora is suitable to act as a "biomarker" reflecting the negative effects of pesticides treatment and is commonly used in eco-toxicological tests to evaluate the influence of these chemicals on soil system (Doelman *et al.,* 1994; Edwards *et al.,* 1990.)

1.7 Impact of Pesticides on Soil microbiota

Soil is the most important site of biological interactions. The indiscriminate use of pesticides disturbs the soil environment by affecting flora and fauna including microflora of the soil, and also the physicochemical properties of soil like pH, salinity, alkalinity leading to infertility of soil.(Mohiuddin *et al*., 2013) The important microflora, beneficial for the growth of plants includes nitrogen fixing bacteria like *Azotobacter*and phosphate solubilizing bacteria and Plant Growth Promoting Rhizobacteria(PGPR) like *Bacillus megaterium , Bacillus pumilus, Pseudomonas fluroscene* and other present in the rhizosphere of the plant. The rhizosphere is the zone of soil surrounding the root which is affected by these microbes. Significance of the rhizosphere arises from the release of organic material from the root and the subsequent effect of increased microbial activity on nutrient cycling and plant growth. Excess application of pesticides like Butachlor, Cypermethrin, Copperoxychloride may

adversely affect the function of the rhizospheric microorganisms .Although, pesticides are intended to protect crops, they may affect non-target organisms and contaminate soil environment altering the equilibrium of soil processes for shorter or longer period.(Mohiuddin *et al*., 2013)

In farms, pesticides affect beneficial soil microbes that may be present in soil. Increases introduction of soil with pesticides can cause decline in beneficial soil microorganisms population. Some example are given below:

- The pesticides like Triclopyr inhibit the growth of soil microbes that transform ammonia into nitrite (Pell *et al*., 1998)
- Glyphosate reduces the growth and activity of free-living nitrogen-fixing bacteria in soil (Santos *et al.*, 1995) and2,4-D reduces nitrogen fixation process conducted by the bacteria that live in the root nodules of bean plants (Arias *et al*., 1993; Fabra*et al.*, 1997).
- Mycorrhizal fungi grow with the roots of many plants and aid in nutrient uptake. These fungi can also be damaged by herbicides in the soil. Two commonly used herbicides Oryzalin and Trifluralin are found to inhibit the growth of some beneficial species of mycorrhizal fungi (South *et al*.,1978**)**.

2.0 REVIEW OF LITERATURE

Research reported across the globe have clarified the ill effects of pesticideson beneficial soil microbiota, some of is as discussed below :

2.1.Effect of Pesticides on Soil microbiota

2.1.1 Effect of fungicide on useful microbes

Fungicides are pesticides that kill or prevent the growth of fungi and their spores . They can be used to control fungi that damage plants.Several studies have reported the harmful effects of fungicides on soilmicrobial growth, survival, and activity (Cyco´ n*et al.*,2006) (Meena*et al.,* 2020). Most copper (Cu) based fungicides have a deleterious effect on the population of N-fixing bacteria (Zwieten*et al.*,2003). Fungicidal residues, for example, Apron, Arrest, Captan, tend to remain in soil reacting with living organisms and affecting the N-fixation in legume-*Rhizobium* association (Boahen*et al.*,2001).

Carbendazim is moderately toxic to *Pseudomonas fluorescens* and *Bacillus subtilis*while being highly toxic to *Trichodermaharzianum*, a potent naturalbiocontrol agent active against soil-borne fungal

phytopathogens (i.e., *Fusarium*, *Pythium*, and *Rhizoctonia*) on soybean, potato, cotton and other crops (Virág*et al.*, 2007).

The impact of fungicides such as Chlorothaloniland Azoxystrobin on soil microbial activities has long been recognizedwith negative effects on the biocontrol agents itself as in Fusarium- wilt (Fravel*et al.*,2005) .The impact of a wide range of fungicides on beneficial soil microbiota is depicted

Table 3 Effect of Fungicides on soil microbiota

S.No.	Pesticide	Targeted soil microbes	Activity	Reference
1	Benomyl	*Pseudomonas fluorescene*	*P.fluorescens* is less sensitive to Thiram	Kurhade*et al.*,2016
2	Captan	Aerobic N-fixing, nitrifying, denitrifying bacteria, Phosphate Solubilizing Bacteria (PSB) and other beneficial fungi	Inhibits the growth of these bacteria and nitrogenase activity	Sáez, F, *et al.*, 2005
3	Captan	*Azospirillumbrasilense*	Reduce cell growth and nitrogenase activity in the bacteria even at a lower dose	Di Ciocco,*et al* .,1997
4	Captan	*Rhizobium ciceri*	Decreases viable counts of Rhizobium ciceri	Boahen, *et al*.,2001
5	Carbendazim	*Azotobacter*, *Azospirillum* , Phosphate Solubilizing Bacteria (PSB)	Negative impact on growth	Marrone*et al.*, 1993
6	Dithianon	All soil microbes	Destroy bacterial diversity in soil	Liebich*et al* ., 2003
7	Mancozab	Impacts on bacteria involved in N & C cycle in soil	Negative impact	C˘ ernohlávková*et al*., 2009
8	Thiram	*Rhizobium japonicum*	Negative effect on growth	Tu *et al*, 1977
9	Thiram	*Mesorhizobiumciceri*	Negative effect on growth	Gaind*et al*, 2007
10	Thiram	*Pseudomonas fluroscen**e***	*P.fluorescens*is less sensitive to Thiram	Kurhade*et al.*, 2016
11	Tebuconazole	*Rhizobium*sp	Negative effect on pea-*Rhizobium* symbiosis by reducing the growth, symbiotic traits, grain yield and nutrient uptake by plants.2121	Ahemad*et al.*, (2011)
12	Mancozab	Impacts on bacteria involved in N & C cycle in soil	Negative impact	C˘ ernohlávková*et al.*, 2009.

2.1.2 Effect of Herbicide on Soil Microbiota

Herbicides are substances used to manipulate or control unwanted vegetation. Herbicide application occurs most frequently in row crop farming to maximize crop productivity byminimizing other vegetation.(US EPA, 2021) Herbicides can act by inhibiting cell division , photosynthesis or amino acid production or by mimicking natural plant growth hormones,causing deformities.(Ross and Childs *et al.*,1996).But the use of herbicides also affects soil biota . Herbicides causes a reduction in the total microbial population in soil within 7 to 30 days after application depending on the type of herbicidal molecules (Milosevic *et al*., 2002) and adversely affect the microbial biodiversity indirectly by altering the physiology or biosynthetic mechanisms (Kremer *et al.*,2009)The application of excessive and higher doses of herbicides has also been reported to result in the death of many sensitive microbes (Milosevic*et al.*,2002).The harmful effect of the chemical herbicides used on the soil microbial diversity depend on the adsorption degradability, bioavailability, bioactivity, persistence, toxicity and concentration of agrochemicals along with soil factors such as texture, vegetation, tillage system, andorganic matter (Hussain *et al.*,2009, ; Datta *et al.*, 2017).The soil type can also play a critical role in the herbicidal effect. The effect can be more severe in coarse-textured soils. For example, (Khan *et al*., 2006) reported severe negative effects of herbicides on microbial association and vitality of chickpea in sandy clay loam soils (Khan *et al.,* 2006). The herbicidal molecules belonging to the Triazines group are more hazardous when applied over a long time due to their residual effect and persistence in soil (Milosevic *et al.*,2002). The repeated applications of Atrazine can significantly reduce the intensity of soil microbes (Seghers *et al* .,2003). Similarly, Atrazine and Metolachlorcan alter the biodiversity of different species of actinomycetes and bacteria in soil (Seghers, *et al* .,2003). The effects of different herbicides on the soil microbiota are presented in Tables which is given below:

Table 4 Effect of Herbicide on soil microbiota

S.No.	Pesticides	Targeted Soil Microbes	Activity	Reference
1.	Glyset I.P.A, Glyphosate 48%	soil bacteria, fungi and actinomycetes	negatively affect microbial counts and activity in soil	Mohiuddin *et al.* ,2013
2.	Glyphosate	*azotobacter*	Reduces the growth and activity of azotobacter	Santos *et al*., 1995

3.	Butachlor	*Azospirillum* and anerobic nitrogen fixers	Negative effect on growth	Jena *et al*., 1987
4.	2,4-dichlorophenoxyacetic acid	*Rhizobium* sp.	Deleterious effect on the growth of *Rhizobium* sp. Causes cell lysis and modified the membrane fluidity in *Rhizobium*	Peretti *et al.*(1992) Fabra *et al* ,1997
5.	2,4-dichlorophenoxyacetic acid	Purple non sulphur bacteria	Reduces nitrogenase, phosphatase and hydrogen photoproduction activities of purple non-sulfur bacteria	Chalam *et al.*, 1997.
6.	2,4-dichlorophenoxyacetic acid	Blue green algae, *Nitrosomonas* and *Nitrobacter*	2,4-D also reduces fixation by blue-green algae and nitrifying process impacting *nitrosomonas* and *Nitrobacter* sp.	Fox *et al.*, 2001
7.	Isoproturon	*Nitrosomonas*, *Nitrobacter*, urea hydrolyzing bacteria, *actinomycetes* and fungi	Adversely impacts *nitrosomonas*, *Nitrobacter*, urea hydrolyzing bacteria, nitrate reductase activity and growth of actinomycetes and fungi	Nowak, *et al* 2002
8.	Metribuzin	*Rhizobium leguminosarum*	negative effect on the lentil (*Lens culinaris*) – *Rhizobium leguminosarum* symbiosis	(Sprout *et al.*, 1992)
9.	Triclopyr	*Nitrosomonas*, *Nitrobacter*, urea hydrolyzing bacteria, actinomycetes and fungi	Adversely impacts nitrosomonas, Nitrobacter, urea hydrolyzing bacteria, nitrate reductase activity andgrowth of actinomycetes and fungi	Nowak *et al.*,2002

2.1.3. Effect of Insecticides on soil microbiota

Insecticides are pesticides that are formulated to kill, harm, repel or mitigate one or more species of insect. Insecticides work in different ways. Some insecticides disrupt the nervous system, whereas others may damage their exoskeletons, repel them or control them by some other means. Because of these factors, each insecticide can pose a different level of risk to non-target insects, people, pets and the soil environment. These chemicals affect soil microbes also. Soil-dwelling microbes can be genetically modified using insecticides in a manner that is no longer helpful to the soil ecosystem and

may eventually become resistant to the chemicals, intended to exterminate them. Insecticides have a higher effect on soil microbes compared to herbicides, albeit less than that of fungicides (Aktar *et al*., 2009). Some insecticides are detrimental to the growth and survival of beneficial microbes, but others may have stimulating or no effects (Patnaik *et al.*,1996). For example, insecticides of the carbamate group (e.g., Carbofuran, Methiocarb, And Carbaryl) have a wide range of negative impact on soil microbial environment (Sannino *et al.,* 2001) and enzymatic activity (Kalam *et al*.,2001). Similarly, insecticides belonging to the chemical group of organophosphates (i.e., Dimethoate, Diazinon, Chlorpyrifos, Quinalphos, And Malathion) inhibit the growth and population of soil bacteria, fungi (Pandey *et al.,* 2004), and enzymes (Singh *et al.*,2005).The applied insecticides affect the growth, survival, and working capacity of symbiotic rhizobialassociationwith roots of legume plants resulting in dwindled atmosphericN-fixation also (Niewiadomska *et al.,* 2004). The effects of different insecticides on the soil microbiota that are linked with nutrient cycling are presented in Tables which is given below:

Table 5 Effect of Insecticide on soil microbiota

S.No.	Pesticides	Targeted soil microbes	Activity	Reference
1	Carbary and Carbofuran	*Azospirillum* and anerobic nitrogen fixers	Negative effect on growth	Jena *et al.*,1987
2	Alphacypermethrin 10%	soil bacteria, fungi and actinomycetes	pesticides negatively affect microbial counts and activity in soil	Mohiuddin *et al*.,2013
3.	Diazinon	*Proteus vulgaris*	Inhibits urease-activity	Ingram *et al*.,2005
4.	Lindane	soil microbes in a *Arachishypogaea* field	Decreases microbial biomass and microbial andenzymatic activities as a result of longer persistence in soil.Lindane inhibit state of nitrification, N-availability,P-solubilization and activity of phosphomonoesterase enzyme	Singh *et al*., 2005
5.	Chlorpyrifos	Soil nitrifying microbes	Reduces ammonification process	Madhuri *et al*., 2002
6.	Chlorpyrifos	Soil nitrifying microbes	Reduces the population of aerobic N-fixing, nitrifying,denitrifying bacteria and several fungi	Martínez *et al*., 1998

2.2 Role of soil bacteria in pesticides degradation

It is reported that some soil bacteria have adapted to changing environment by evolving the mechanism of pesticide degradation. Here some soil bacteria spp. is given which play an importantrole in pesticides degradation.

Table 6 Pesticide degradation by soil microbiota

S. No.	Soil microbes	Degraded pesticides	Type of pesticides	Reference
1	*Pseudomonas putida*	Fenamiphos (FEN)	Organophosphate	Chanika *et al* ., 2011
2	*Acinetobacterrhizosphaerae*	Fenamiphos (FEN)	Organophosphate	Chanika *et al* ., 2011
3	*Bacillus megaterium*	Chlorpyrifos-Methyl	Organophosphate	Zhu *et al.,* 2019
		Mesotrione	Organophosphate	Bardot *et al*;2015
4	*Bacillus subtilis*	Chlorpyrifos	Organophosphate	Helow *et al* .,2013
5	*Bacillus pumilus*	P-Nitrophenol	Organophosphate	Ali *et al.,* 2012
		Chlorpyrifos	Organophosphate	Anwar *et al* .,2009
		Malathion	Organophosphate	Hussaini *et al*., 2013
		Dimetoate	Organophosphate	Hussaini *et al.,* 2013

2.3 Soil-borne bacteria and their relevance

In soil thousands of bacterial species are found which play an important role in rhizosphere and soil environment, the role and relevance of many have been clearly defined and established. Bioprospecting for probable and potent stress resistant species has been a subject of concern in the recent past. Given the uncontrolled usage of pesticides and the increasing levels of pesticide residue in soil and groundwater, focus now is to screen for microbial species that could exhibit pesticide degradation calibre. Among the many researched bacterial species, a few, namely, *Bacillus megaterium, Bacillus pumilus, Pseudomonas fluorescens* have gained relevance in the recent past.

2. 3.1 Role and relevance of *Bacillus megaterium*

2.3.1.1 Systematic

Kingdom : Bacteria

Phylum : Firmicutes

Class : Bacilli

Order : Bacillales

Family : Bacillaceae

Genus : Bacillus

Species : *B. megaterium*

2.3.1.2 Morphology

Bacillus megaterium is a gram positive and rod shaped bacterium, and produces endospores, plant growth promoting substances and inhibitory molecules. Bacterium found in soil, seawater, sediments, rice paddies, dried food, milk. (Vary*et al.*,1994)

2.3.1.3 Culture characteristic

B. megaterium grows at temperatures from 3 °C to 45 °C, with the optimum around 30 °C. (Vos*et al.*,2009) required pH 7.0. Plates are incubated at 30 °C. colony is concave ,smooth andmilky white in color.

2.3.1.4 Recent research

a) *B. megaterium* bacterium has recombinant protein production capacity (Bunk*et al.*, 2010).
b) *B. megaterium* is used in industry to produce vitamin B12 and penicillin amidase and its use in AIDS diagnostics (Vary*et al.*, 1994.; Ginsburgh*et al*., 1989).
c) *B. megaterium* produces metabolites with antibacterial and antifungal activities (Thubiani*et al*., 2018).Behenic acid, β-sitosterol, and phenylacetic acid are natural antimicrobial compound found in *B. megaterium* strain L2 .(Xie *et al.*, 2021).
d) *B.megaterium* is a Plant Growth Promoting Rhizobacteria(PGPR) influence plant growth and development by the production of phytohormones such as cytokinins and cytokinin receptors play a complementary role in plant growth promotion by *B. megaterium. (*Castro *et al.*, 2008)
e) *B. megaterium* mj1212 treatment act as phosphate biofertilizer to improve the plant growth.(Kang *et al.*, 2014)
f) *Bacillus megaterium* de Bary TRS-4 markedly reduce brown root rot disease ,caused by *Fomeslamaoensis* in *Camelliasinensis* (Chakraborty *et al.*, 2006).

g) *Bacillus megaterium* to prevent the outbreak of this disease caused by *Alternaria japonica* in the crop of *Brassica oleracea var. italica* (Ramiro Daniel AcurioVásconez, Biotechnology Reports ,2020)

h) The edaphic *Bacillus megaterium* strain Mes11 can degrade the herbicide mesotrione. (Bardot *et al.*,2015)

i) *Bacillus megaterium* CM-Z19 92.6% degrade chlorpyrifos-methyl (100 mg/L) within 5 days of incubation. CM-Z19 has high phosphoesterase activity and ability to degrade the organophosphorus pesticide also. (Zhu *et al*., 2019)

2.3.2 *Bacillus pumilus*

2.3.2.1 Systematics

Kingdom : Bacteria

Phylum : Firmicutes

Class : Bacilli

Order : Bacillales

Family : Bacillaceae

Genus : Bacillus

Species : *B. pumilus*

2.3.2.2Morphology

*Bacillus pumilus i*s a spore-forming bacteria that is rod-shaped, Gram-positive, and aerobic. It resides in soils and some colonize in the root area of some plants.

2.3.3.3 Culture characteristic

B. pumilus colonies are opaque and off-white. They are roughly circular, but with an irregular margin, and have a slight filamentous appearance.

2.3.3.4 Recent research

a) *B. Pumilus* shows high resistance to environmental stresses, including UV light exposure, desiccation, and the presence of oxidizers such as hydrogen peroxide. (J. Craig Venter Institute, 2011)

b) *B. pumilus*SQR-N43 is a potent antagonist against *R.solani* Q1 which produces damping off disease in cucumber(Huang *et al.*,2012).

c) *Bacillus pumilus* is a plant-growth-promoting rhizobacteria (PGPR).It has a strong growth-promoting activity by the production or accumulation of bioactive C19gibberellins. (Francisco Javier GutiérrezMañero,*et al*, 2001)

d) In wheat, *B. Pumilus* also induces plant resistance to Take-all (*Gaeumannomyces graminis*), a fungal disease which can significantly damage wheat crops (Sari *et al*., 2007). Additionally, *B. pumilus* is thought to function as a plant growth promoting endophyte in *Vitisvinifera* plants .

e) *Bacillus pumilus*C2A1 is effective in degrading chlorpyrifos and its first hydrolysis metabolite is 3,5,6-trichloro-2-pyridinol (TCP).The strain C2A1 shows 90% degradation of TCP (300 mg L−1) within 8 days of incubation.(Anwar*et al.*, 2009)

f) *Bacillus pumilus*, shows effective degradation of Malathion and Dimetoate with 45% and 37% degradation respectively (Hussaini *et al.,* 2013).

g) *B. pumilus*strain W1 degrade organophosphate pesticide p-nitrophenol and a gene opdA, a gene found in this strain, play role in the degradation of p-nitrophenol.(Ali *et al.,* 2012)

h) *B.pumilus*strain TW-2 possessed strong inhibition on ear neck blast of field rice and good control effect on blast. (Guimei *et al*., 2008)

i) *B. pumilus* K1 was able to metabolize Isoproturon (IPU) effectively and thus could be employed for development of field scale bioremediation technology. Isoproturon is a herbicide. (Giri*et al*., 2017)

j) *B. pumilus* can be considered as a very promising biocontrol agent as it's strain 344-3 of *B. pumilus* 344-3 culture supernatant application on the surface of maize kernels provided 99% reduction in ochratoxin A (OTA) production potential of *Aspergilluscarbonarius*AC82.(Higazy *et al.*, 2021)

2.3.3 *Pseudomonas fluorescens*

2.3.3.1 Systematic

Domain : Bacteria

Phylum : Proteobacteria

Class : Gammaproteobacteria

Order : Pseudomonadales

Family: Pseudomonadaceae

Genus : Pseudomonas

Species : *P. fluorescens*

2.3.3.2 Morphology

It is an obligate aerobe, gram negative rod shape bacteria. These bacteria are able to inhabit many environments, including: plants, soil, and water surfaces. Its name comes from its production of the soluble fluorescent pigment pyoverdin.

2.3.3.3 Culture characteristic

*Pseudomonasfluorescens*colony is convex shape, smooth edge and cream in color.

2.3.3.4 Recent research

a) *Pseudomonasfluorescens* are common soil bacteria that can improve plant health through nutrient cycling, pathogen antagonism and induction of plant defense. (silby *et al.,* 2009)
b) *Pseudomonasfluorescens* strains obtained from the rhizosphere of different crops and strains that show inhibitory action against the chickpea (*Cicerarietinum*) wilt pathogen *Fusariumoxysporumf. sp. ciceris*.(Vidhyasekaran *et al.,* 1995)
c) *Pseudomonasfluorescens* Pf-5 is a plant commen soil bacterium that inhabits the rhizosphere and produces secondary metabolites that suppress soilborne plant pathogens. (Paulsen *et al*., 2005)
d) *Pseudomonasfluorescens* strain CHAO suppresses *Thielaviopsisbasicola*-induced black root rot of tobacco by production of 2,4-diacetylphloroglucinol, a metabolite with antifungal, antibacterial, and phytotoxic activity. (Keel *et al.,* 1992)
e) Massetolide A is a cyclic lipopeptide (CLP) antibiotic produced by various Pseudomonas fluorescens strains from diverse environments.(Bruijn *et al*.,2008)
f) *Pseudomonas fluorescens*act as plant growth promoting rhizobacteria as it increases seed germination percentage, root length, shoot length, fresh weight, dry weight and vigour index in cotton field.(Shanmugaiah *et al*., 2009)

g) Immobilized cells of P. fluorescens SM1 strain is quite effective in bioremediation of major toxicants Cu++, Cd++, Ni++, and Pb++ present in Indian water bodies. (Wasi*et al.*, 2011)

h) *P.fluorescens* is less sensitive to Thiram @ 0.2% (2.15 × 108 cfu/ml), Benomyl @ 0.3% (1.63 × 108 cfu/ml) and Carbendazim @ 0.1% (1.38 × 108 cfu/ml).(Kurhade *et al.*, 2016).

2.4 Relevance of Chosen Pesticides

2.4.1 Cypermethrin

- It is most consumed pesticides in country (http://indiaforsafefood.in/farminginindia/)
- Broad-spectrum Insecticide and pyrethroid type pesticide.
- Causes oxidative stress. (Huang, *et al*., 2016)
- Causes liver damage. (Priyanka *et al*.,2020)
- Highly toxic to fish, bees, and aquatic insects. (NPTN)
- Carcinogenic. (US EPA)

2.4.2 Copper- oxychloride

- Mostly used pesticide in country. (http://indiaforsafefood.in/farminginindia/)
- It is a broad spectrum fungicide.

2.4.3 Butachlor

- Most consumed herbicide in India. (http://indiaforsafefood.in/farminginindia/)
- It can retard growth and cocoon production and cause damage to epithelial tissue in earthworm. (Muthukaruppan Gobi *et al*.,2009)

3.0 OBJECTIVE

1. To study the degradation of Cypermethrin (insecticide) using *Bacillus megaterium*, *Bacillus pumilus*, and *Pseudomonas fluorescens.*
2. To study the degradation of Copper-oxychloride(fungicide) using *Bacillus megaterium*, *Bacillus pumilus*, and *Pseudomonas fluorescens.*
3. To study the degradation of Butachlor (herbicide) using *Bacillus megaterium*, *Bacillus pumilus*, and *Pseudomonas fluorescens*

4.0 PLAN OF STUDY

Phase 1

a) Procurement of Culture

b) Identification and preservation of culture

Phase 2 Applications of Pesticides on Bacterial Culture

Phase 3 Growthpatternstudyof bacterial culture in pesticides containing culture and in control Culture.

Phase 4 Observation of microbial activity in pesticides containing culture

Phases 5 Study the degradation of pesticides.

5.0 METHODOLOGY

1. Procurement of culture – Cultures of *Bacillus* spp and *Pseudomonas* spp procured from USDA(United States Department of Agriculture).

2. **Preservationandmaintenanceofculture –**Cultures will be preserved as Agar Slants and Glycerol Stock. They will be serially Sub-cultured from time to time for further use..

3. Effect of pesticides on bacterial culture – Different pesticides will be added in different bacterial culture. Standard Plate Count Method will be use for estimating the culture concentration.

4. Pesticides degradation by bacterial culture-Pesticides degradation is measured by technique like High pressure liquid chromatography (Kim *et al* .,2010)or gas chromatography(James *et al*.,1952)

6.0 EXPECTED OUTCOME

By studying the effect of the above pesticides on the above bacterial species we will be able to know whether these pesticides is affecting growth and activities of these bacterium species positively or negatively or remain unaffected and if these bacteria breakdown /degrade pesticides we will be able to use them in agriculture because these are agro- friendly bacteria.

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